Ann. For. Res. 66(1): 1258-1272, 2023
ISSN: 18448135, 20652445

ANNALS OF FOREST RESEARCH
www.e-afr.org

EFFECT OF SOME PESTICIDES ON 1-AMINOCYCLOPROPANE-1-CARBOXYLATE (ACC) DEAMINASE BIOSYNTHESIS GENE OF DROUGHT RESISTANCE *PSEUDOMONAS PUTIDA* AND TRANSCONJUGANT *PSEUDOMONAS FLUORESCENS*

Karzan Kazm Mahmud[1*], Aras Muhammad Khudhur[2], Goran Noori Saleh[3], Muzhda Haydar Saber[4], Sakar B. Smail[6] and Abdulkarim Yasin Karim[5]

[1]Department of Plant Protection, College of Agricultural Engineering Sciences, University of Salahaddin, Erbil, Kurdistan Region, Iraq

[2]Department of Soil and Water, College of Agricultural Engineering Sciences, University of Salahaddin, Erbil, Kurdistan Region, Iraq

[3]Department of Nursing, faculty of Nursing, Tishk International University, Erbil, Kurdistan Region, Iraq

[4]Department of Medical Analysis, Faculty of Applied Science, Tishk International University, Erbil, Kurdistan Region, Iraq

[5]Department of Microbiology, PAR Private Hospital, Erbil, Kurdistan Region, Iraq

[6]Department of Biology, College of Science, University of Salahaddin, Erbil, Kurdistan Region, Iraq

[6]Department of Community Health Nursing, College of Health Technology, University of Cihan, Erbil, Kurdistan Region, Iraq

***Corresponding author:**

Karzan Kazm Mahmud

Email: karzan.mahmud@su.edu.krd

Abstract

This research was conducted to evaluate the effects of four main usable pesticides in Kurdistan Region – Iraq on plant growth promoting and drought resistance bacteria *Pseudomonas putida* and transconjugant *Pseudomonas fluorescens* contained 1-aminocyclopropane-1- carboxylate (ACC) deaminase gene. The most usable pesticides in the region were Glyphosate, Topsin, Vantex and Velum prime used at the recommended concentrations in bacterial culture media and soil. According to the results, herbicide Glyphosate did not have notable effects on the both bacterial numbers and viability of 1-aminocyclopropane-1- carboxylate (ACC) deaminase gene in the genomes of *Pseudomonas putida* and transconjugant *Pseudomonas fluorescens*. However, other pesticides were exhibited inhibitory effects on the growth of tested bacteria in culture media and soil. Furthermore, negative effects were revealed on the viability of the gene after treating with Vantex and Velum prime pesticides in culture media. While, there was the gene viability in *Pseudomonas putida* genomes treated with Topsin in culture media and soil. The results illustrated

© ICAS 2023

that Glyphosate does not have significant effects on *Pseudomonas putida* and transconjugant *Pseudomonas fluorescens* bacterial communities and genomes, whereas the other three pesticides have negative impacts on the both bacterial communities and both bacterial genomes especially Vantex and Velum prime. Therefore, it has been suggested that using Topsin, Vantex and Velum prime pesticides should be limited or avoided for controlling plant fungi, insects and nematodes respectively.

Keywords: *Pseudomonas putida* strain UW4, Transconjugant *Pseudomonas fluorescens*, Pesticides, Bacterial numbers, ACC deaminase gene.

Introduction:-

Pesticides are the most usable chemical substances in the Kurdistan region - Iraq that are used widely for controlling unwanted weeds, plant pathogens and pests. Pesticide is a combination of chemical substances which are mostly included fungicides, insecticides, nematocides and herbicides (Grube et al., 2011). It is mentioned that approximately 80% of the chemical substances in the world are utilized as pesticides for controlling some agro-pathogens and pests (Muturi et al., 2017). In several researches illustrated that soil microorganisms are able to grow in the presence of many pesticides. The process of catabolism and detoxification metabolism may happen during the utilization of the pesticide as a carbon and energy sources by soil microorganisms. However, they are probably toxic substances for the microbial habitats due to the impacts directly or indirectly on the structures and functions of the some microorganisms in the soil (Grube et al., 2011). Although, some bacterial species may use pesticides as sources of nutrients for their growth and replication, there are huge of bacterial species that are susceptible, and probably manipulated their genomes or decimated by pesticides (Russell et al., 2011). AL-Ani et al., (2019), demonstrated that Glyphosate, Malathion and Alphacypermethrin pesticides had negative impacts on the reducing of the microbial activities and numbers of soil bacteria, fungi and actinomycetes.
It is highlighted that some pesticides in high doses can cause free radicals to produce reactive oxygen species (ROS), which have ability to damage cellular pathways through inactivation different receptors and enzymes. Additionally, pesticides can promote DNA adducts, oxidative DNA damage and dsDNA or ssDNA fractures (Kaur and Kaur, 2018). Pesticides also increase the mutation of bacterial genomes, as pesticides enhance bacterial stresses and caused acquisition of mobile gene elements through different mechanisms such as; inhibition of outer membrane pores, activation of efflux pumps and gene mutation induction (Qiu et al., 2022).
There are some species of *Pseudomonas* contain effective 1-aminociclopropane-1-carboxylase (ACC) deaminase enzyme. This enzyme has ability to modify and break ACC into ammonia and α-ketobutyrate (Zarei et al., 2020). Therefore, ACC deaminase enzyme is one of the important enzymes that are capable of decreasing ethylene of plants under biotic and abiotic stress conditions. Thus, treating plants with the bacteria that express this enzyme is the most efficient mechanisms to promote plant tolerance to dissociation stress. Moreover, this enzyme has ability to induce plant roots to be taller, and to be more resistant to growth inhibition by a variety of ethylene-inducing stresses (Mahmud and Khudhur, 2022).

© ICAS 2023

The ACC deaminase gene (*acdS*) is one of the integrative and conjugative gene elements, and it has been used recently in horizontal conjugation gene transfer techniques (Mahmud, 2022). *Pseudomonas putida* strain UW4 contain the most effective ACC deaminase gene compared with other strains of bacteria. Thus, ACC deaminase gene in this strain has been transferred into *Pseudomonas fluorescens* by conjugation and molecular cloning techniques to generate transconjugant *Pseudomonas fluorescens*, and then used as biofertilizers to increase drought resistance of treated wheat crops (Mahmud, 2022).

This study aimed, therefore, to evaluate the effects of four main usable pesticides in Kurdistan Region – Iraq on plant growth promoting and drought resistance bacteria (*P. putida* and transconjugant *P. fluorescens*) contained 1-aminocyclopropane-1- carboxylate (ACC) deaminase gene. The most usable pesticides in the region were Glyphosate, Topsin , Vantex and Velum prime used at the three different concentrations in culture media and soil to evaluate the impacts of the mentioned pesticides on the selected bacterial numbers and availability of ACC deaminase gene (*acdS*) in the bacterial genomes.

Materials and Methods:

Preparation of bacteria and pesticides

Both bacteria in this study (*P. putida* strain UW4 and transconjugant *P. fluorescens*) contained ACC deaminase genes (*acds*) were provided from a PhD. research study. They were identified by VITEK-2 compact and molecular identification techniques (Mahmud, 2022). The pesticides which were used in this study were fungicide (Topsin), insecticide (Vantex), nematocide (Velum prime) and herbicide (Glyphosate).

Screening the effects of the pesticides on the bacteria in culture media

The provided bacteria were re-cultured in nutrient agar media at 28°C ±2 for 48 hours to confirm viability, and then single colony of each bacterium was cultured into 10mL of liquid broth media for serial dilution and bacterial plate counting methods. Three different concentrations were prepared from each pesticides based on the concentrations that were used regularly in the Kurdistan region by farmers. The concentrations were 5µL, 10µL and 15µL of each pesticide in 10mL of cultured liquid broth media that contained approximately (6.9×10^6 CFU/mL) of the bacteria. Serial dilution and bacterial plate counting methods were performed for each treatment to re-count the numbers of viable bacteria after treating with the pesticides, and compared with the controls for revealing the effects of the pesticides on the bacterial numbers in culture media.

Screening the effects of pesticides on the bacteria in soil

Three different concentrations of each pesticide were prepared which were 5µL, 10µL and 15µL of each pesticide in 10mL of sterilized D.W. The growth bacteria from the stock culture were re-numbered to around (6.4×10^6 CFU/mL), and mixed in sterilized petri dishes contained 15g of sterilized SULIFLOR pittmoss soil which is rich in nutrient. Then, the prepared concentrations of the pesticides were disseminated on the prepared petri dishes except for the controls. All treatments were incubated at 30°C ±2 for three days. Then, isolation, purification and screening the numbers of viable bacteria were conducted in each treatment by serial dilution and bacterial plate counting

© ICAS 2023

techniques. Statistically, the data were analyzed to know the effects of the pesticides on the number of the bacteria in soil.

ACC deaminase gene viability screening

In order to know the availability of ACC deaminase genes in the genomes of all treated bacteria with pesticides, the viability of treated bacteria in both experiments (treated bacteria with the pesticides in culture media and soil) were cultured in liquid broth media at 28°C ±2 for 48 hours. The genomes of the growth bacteria were extracted according to the PureLink Genomic DNA procedures. Quantity and quality of the extracted genomes were confirmed via NanoDrop technique.

The primers for the ACC deaminase gene in the bacteria were selected and designed by [National Center for Biotechnology Information (NCBI). The primers were; ACC-Forword-5'CGCGCGCATATGATGAACCTGAATCGTTTTGAACGTTATCCG-3'; ACC-Reverse-5'-GCGATACTCGAGTCAGCCGTTGCGAAACAAGAAGCT-3', and the binding sizes were (1017bp). PCR technique was run to bind and amplify the genes in the isolated bacterial genomes. 20µL reactions for PCR were prepared, and the annealing temperature for binding primers with ACC deaminase gene was selected based on the primer melting temperature (Tm). The protocol was consisted from three stages. The first stage was 95ºC for 3 minutes as initial denaturation. The second stage consisted from 95ºC for 30 seconds as denaturation stage, 57ºC for 30 seconds as annealing stage. The third stage was 72ºC for 30 seconds as elongation stage. Eventually, final elongation was conducted at 72ºC for 3 minutes, and the samples were kept at 4ºC until needed. 1% '(w/v)' agarose gel was prepared and run to visualize the binding gene sizes. Amplified ACC deaminase gene was provided and used as positive controls during running agarose gel to compare amplified gene sizes (Mahmud and Khudhur, 2022).

Results and Discussions:

The effects of Glyphosate, Topsin, Vantex and Velum prime pesticides on the numbers of *P. putida* and transconjugant *P. fluorescens* were evaluated in culture media and soil. The results showed that the selected herbicide (Glyphosate) had no significant effects on tested bacterial counts in culture media and soil compared with controls (Figures 1 and 2). The reasons behind of the above results might be due to consume Glyphosate as a nutrient for growth by some soil bacteria, as it was mentioned that some species of *Pseudomonas* sp. have ability to degrade Glyphosate and used them in bioremediation of soil that polluted by this herbicide (Dick and Quinn, 1995; Firdous et al., 2020). Further, it was highlighted that *Pseudomonas sp*. strain CMA 6.9 was isolated from a tank that contained herbicide, and this could be due to its metabolic and physiological versatility (Melo et al., 2017; Lima et al., 2020).

The results of treating both bacteria with fungicide (Topsin) in culture media with different concentrations illustrated significant effects on the bacterial numbers, and their growth were sharply decreased compared to controls especially in 15µL/10mL concentrations (Figure 1). This result was correlated with the results of using fungicide Topsin on *E. coli* counts, as the bacterial numbers were reduced after treating with this fungicide in three different concentrations (Aldehamee, 2015). Our results also are supported by Gallori et al., (1991), Revellin et al., (1993),

© ICAS 2023

Taiwo and Oso, (1997) and Dunfield et al., (2000) who reported that bacterial growth inhibition by fungicides was due to agrochemicals, and they contain similar active ingredient that reduced the numbers of bacteria. Similar trend was reported by (Mårtensson, 1992) who observed that the fungicide treatment decreased the numbers of soil bacteria.

Studied bacterial counts which were treated with insecticide (Vantex) in culture media were reduced gradually from high to low according to the Vantex concentrations, and overall decreased significantly compared with controls (Figure 1). Additionally, few bacterial colonies of both bacteria which were treated with Vantex in the soil were noticed. However, they were out of range (30-300) bacterial colonies based on the bacterial plate counting methods (Figure 2) (Reasoner, 2004). Therefore, they were not counted as bacterial growth numbers. The results of this study are in agreement with previous studies, that is, the analyzed strains of *Pseudomonas* are sensitive to insecticides and inhibition of growth observed when compared with the result of herbicides application. Additionally, it was found that there were variable effects of insecticides on the growth of *Pseudomonas* bacteria (Sudhakar et al., 2000).

Bacterial numbers which treated with nematocide (Velum prime) in culture media were dropped down sharply in all concentrations compared with controls (Figure 1). Furthermore, there were out of range (30-300) of bacterial colonies from both bacteria which were treated with this nematocide in all concentrations in soil (Figure 2). These results indicated that this pesticides had effects on the bacterial growth, as pesticides are able to inhibit cell division through interrupting tubulin and microtubules that detaches cell chromosomes during cells division, and this could be a potential fact for the bacterial binary fission due to have some similarities between prokaryotic and eukaryotic cell divisions (Stenersen, 2004; Eswara and Ramamurthi, 2017).

© ICAS 2023

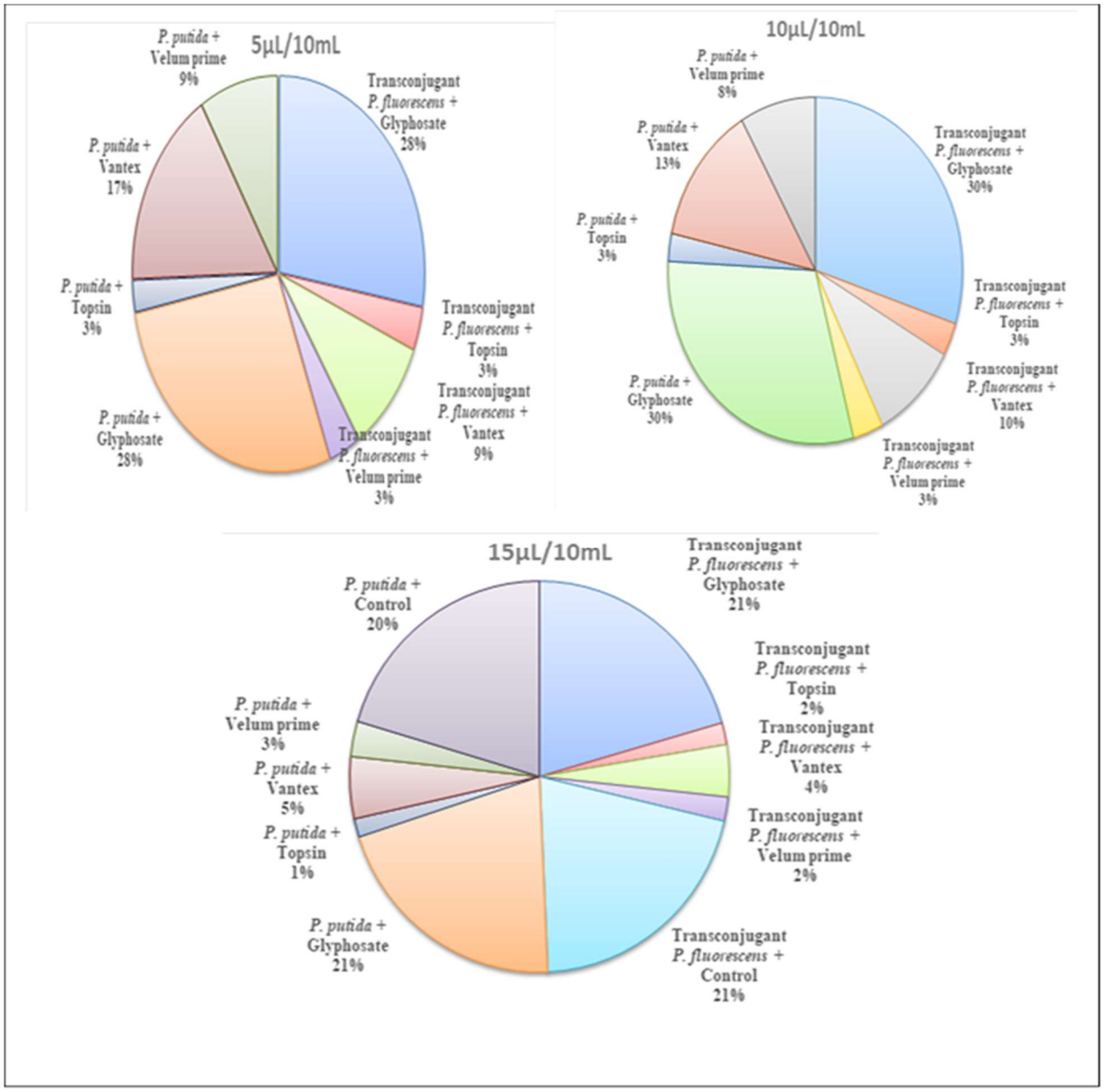

Figure 1: Culture media treatments of both bacteria treated with three different concentrations of the pesticides. Numbers of bacteria in different concentrations CFU/mL.

© ICAS 2023

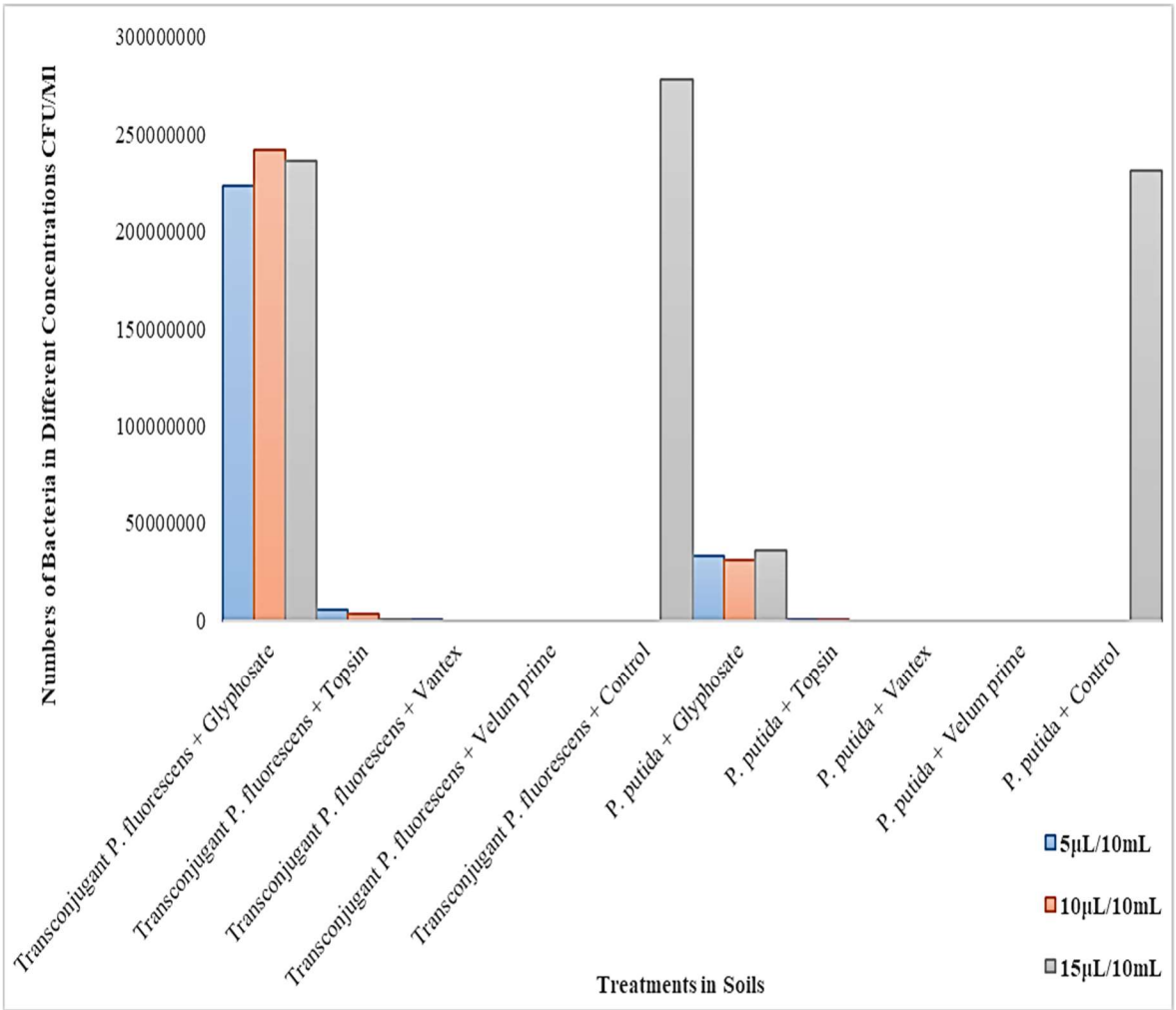

Figure 2: Soil treatments of both bacteria treated with three different concentrations of the pesticides. Numbers of bacteria in different concentrations CFU/mL.

To evaluate the effects of the pesticides on the viability of ACC deaminase gene in both bacteria, molecular identification techniques were carried out. The expected size of ACC deaminase gene is approximately 1017bp based on the used primers. Both bacteria which were treated with Glyphosate had ACC deaminase gene yet in their genomes especially in culture media treatments, as the binding sizes in all concentrations were almost 1017bp (Figure 3, A). In addition, all treatments of both bacteria with Glyphosate in soil had ACC deamianse gene binding size except transconjugant *P. fluorescens* + Glyphosate treatment in concentration 15µL/10mL (Figure 3, B).

© ICAS 2023

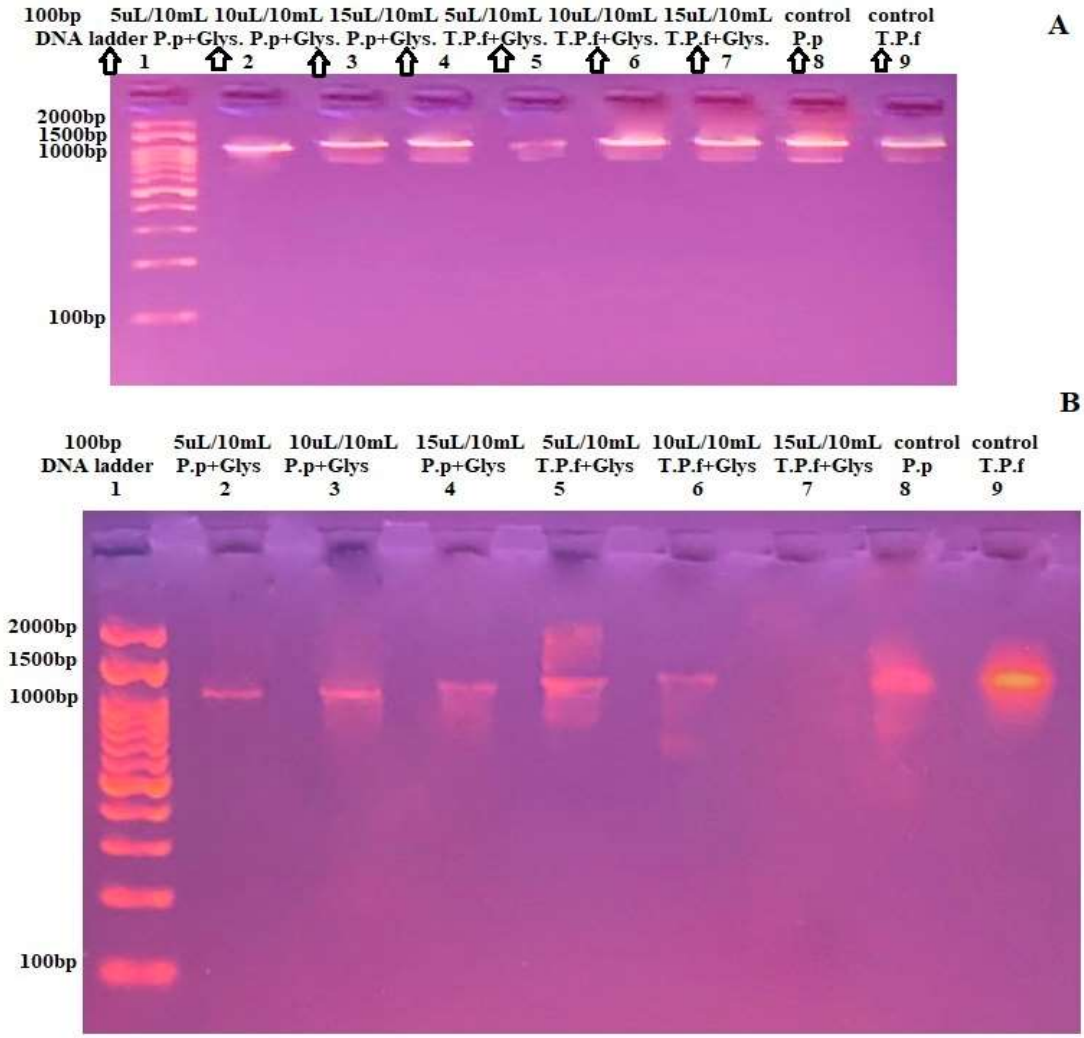

Figure 3: 1% '(w/v)'agarose gel with Ethidium bromide for visualizing ACC deaminase gene. A-Lane 1, 100bp DNA Ladder. A-Lanes 2, 3 and 4, PCR products of ACC deaminase gene from *P. putida* genomes treated in culture media with Glyphosate in 5µL, 10µL and 15µL / (10mL) concentrations respectively. A-Lanes 5, 6 and 7, PCR products of ACC deaminase gene from transconjugant *P. fluorescens* genomes treated in culture media with Glyphosate in 5µL, 10µL and 15µL / (10mL) concentrations respectively. A-Lanes 8 and 9, PCR products of ACC deaminase genes from controls of *P. putida* and transconjugant *P. fluorescens* genomes respectively in culture media. B- Lane 1, 100bp DNA Ladder. B-Lanes 2, 3 and 4, PCR products of ACC deaminase genes from *P. putida* treated in soil with Glyphosate in 5µL, 10µL and 15µL / (10mL) concentrations respectively. B-Lanes 5, 6 and 7, PCR products of ACC deaminase gene from transconjugant *P. fluorescens* genomes treated in soil with Glyphosate in 5µL, 10µL and 15µL / (10mL) concentrations respectively. B-Lanes 8 and 9, PCR products of ACC deaminase genes from controls of *P. putida* and transconjugant *P. fluorescens* genomes respectively soil.

© ICAS 2023

These results demonstrated that Glyphosate does not have effects on the studied bacterial genomes in culture media, whereas in high doses might have effects on the genomes of transconjugant *P. fluorescens* in soil. This could be due to the lack of sufficient nutrients in soil compared with culture media, as bacterial DNA replications and transcription processes require sufficient vitamins and minerals (Hibberd et al., 2017). Further, cell exposing to high doses of chemical substances can cause genome instability and epigenetic modulation with aiding of some environmental factors (Ren et al., 2017). Specifically, it was mentioned that Glyphosate had effects on promoting DNA damage in leucocytes such as PBMCs and cause DNA methylation in human cells in high doses (Kwiatkowska et al., 2017). Therefore, this result concluded that Glyphosate may not have severe effects on the bacterial genomes in culture media, whereas there is possibility to have effects on bacterial genomes in high concentrations especially in soil.

Effects of fungicide Topsin on the availability of ACC deaminase gene in both bacterial genomes were evaluated in culture media and soil. The results illustrated that there were no effects of this fungicide on the ACC deaminase gene availability in all concentration treatments of *P.putida* genomes in culture media (Figure 4, A). While, ACC deaminase gene were unavailable in all concentration treatments of transconjugant *P. fluorescens* genomes treated with Topsin in culture media (Figure 4, A). There were ACC deaminase gene in the genomes of *P.putida* treated in soil with Topsin in concentrations (5µL/10mL and 10µL/10mL) (Figure 4, B). However, there was out of range of *P.putida* colony growth in the concentrations (15µL/10mL) treated with Topsin in soil in order to be ensured the availability of ACC deaminase gene (Figure 4, B). The genomes of transconjugant *P. fluorescens* treated with Topsin in soil were examined for viability of ACC deaminase gene. There was viability of this gene in concentration (5µL/10mL) (Figure 4, B). While, there were no binding gene sizes in concentrations (10µL/10mL and 15µL/10mL) (Figure 4, B).

© ICAS 2023

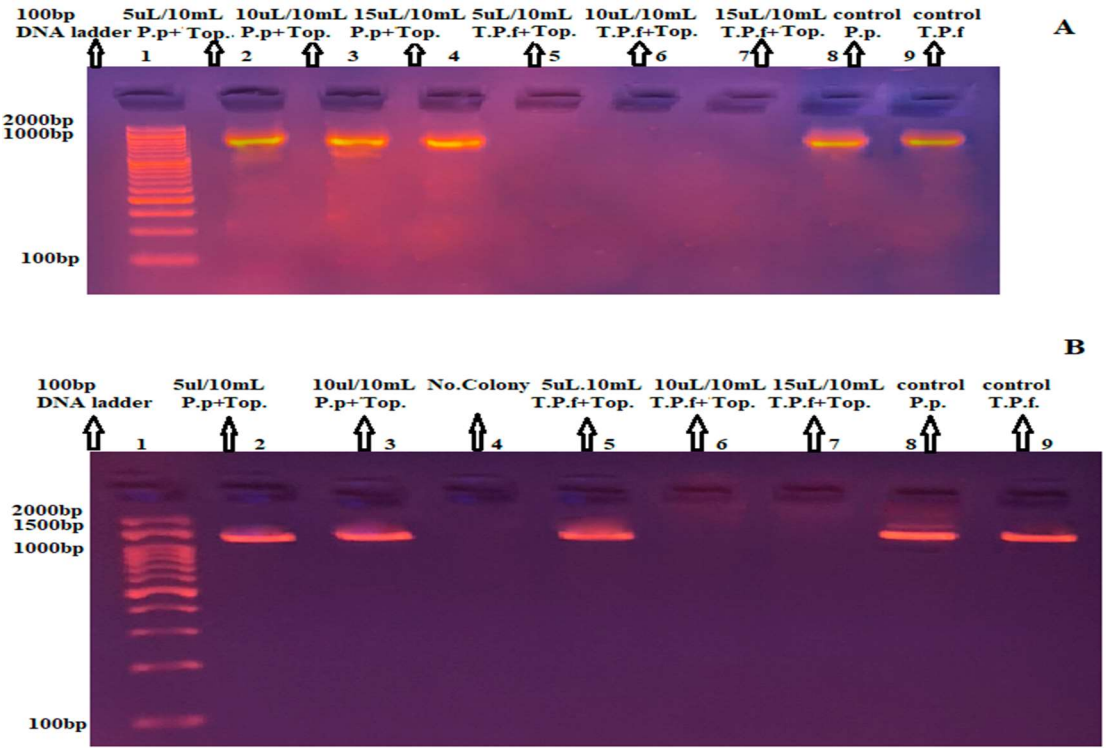

Figure 4: 1% '(w/v)'agarose gel with Ethidium bromide for visualizing ACC deaminase gene. A-Lane 1, 100bp DNA Ladder. A-Lanes 2, 3 and 4, PCR products of ACC deaminase gene from *P. putida* genomes treated in culture media with Topsin in 5µL, 10µL and 15µL / (10mL) concentrations respectively. A-Lanes 5, 6 and 7, PCR products of ACC deaminase gene from transconjugant *P. fluorescens* genomes treated in culture media with Topsin in 5µL, 10µL and 15µL / (10mL) concentrations respectively. A-Lanes 8 and 9, PCR products of ACC deaminase genes from controls of *P. putida* and transconjugant *P. fluorescens* genomes respectively in culture media. B- Lane 1, 100bp DNA Ladder. B-Lanes 2 and 3, PCR products of ACC deaminase genes from *P. putida* treated in soil with Topsin in 5µL and 10µL / (10mL) concentrations respectively. B-Lanes 5, 6 and 7, PCR products of ACC deaminase gene from transconjugant *P. fluorescens* genomes treated in soil with Topsin in 5µL, 10µL and 15µL / (10mL) concentrations respectively. B-Lanes 8 and 9, PCR products of ACC deaminase genes from controls of *P. putida* and transconjugant *P. fluorescens* genomes respectively in soil.

According to the fungicide Topsin treatments, the ACC deaminase gene in the genomes of both bacteria was affected especially in transconjugant *P. fluorescens* treatments. This could be due to the type of fungicide which is systematic. This type of fungicide is absorbable chemical substances that has ability to enter tissues and cells, and interact with the genomes of absorbed cells (Keon et al., 1991). Additionally, Topsin fungicide is capable of interacting with plant cuticle and cell walls physiologically and genetically as well as there are few similarities physiologically between plant and bacteria cell walls. Therefore, this is a potential that this fungicide affected bacterial cells genetically and physiologically through cell walls especially transconjugant *P. fluorescens*, as it

© ICAS 2023

was manipulated previously through entering ACC deaminase gene by horizontal conjugation gene transfer techniques (Cools and Hammond-Kosack, 2013; Szymańska-Chargot et al., 2011).

As has been mentioned from figure (2), there were out of range of both bacterial growth colonies treated with insecticide (Vantex) and nematocide (Velum prime) in soil. Hence, molecular identification of ACC deaminase gene availability was only implemented for the bacteria that were treated with both pesticides in culture media. The results indicated that there was ACC deaminase gene availability in the genomes of *P. putida* treated with Vantex and Velum prime only in concentration (5µL/10mL) (Figure 5, A and B). Additionally, there was ACC deaminase gene availability in the genome of transconjugant *P. fluorescens* treated with Velum prime only in concentration (5µL/10mL) (Figure 5, A and B).

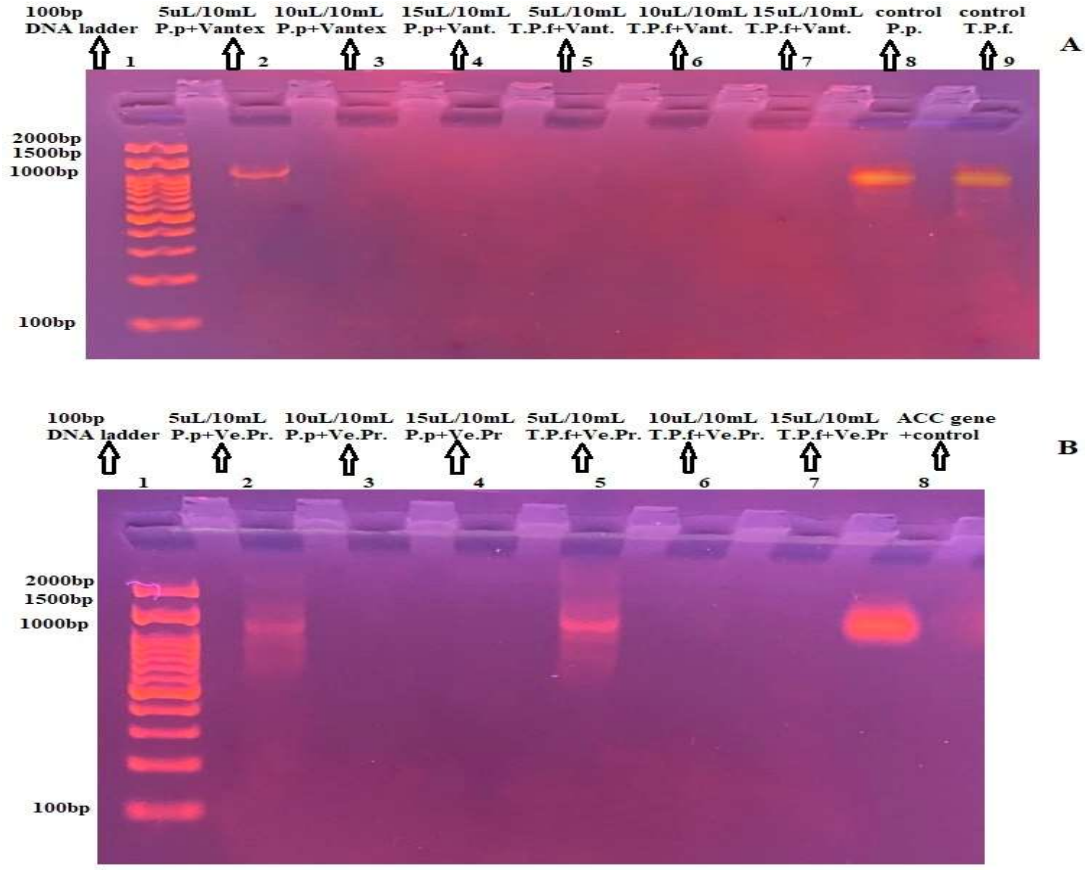

Figure 5: 1% '(w/v)'agarose gel with Ethidium bromide for visualizing ACC deaminase gene. A-Lane 1, 100bp DNA Ladder. A-Lanes 2, 3 and 4, PCR products of ACC deaminase gene from *P. putida* genomes treated in culture media with Vantex in 5µL, 10µL and 15µL / (10mL) concentrations respectively. A-Lanes 5, 6 and 7, PCR products of ACC deaminase gene from transconjugant *P. fluorescens* genomes treated in culture media with Vantex in 5µL, 10µL and 15µL / (10mL) concentrations respectively. A-Lanes 8 and 9, PCR products of ACC deaminase genes from controls of *P. putida* and transconjugant *P. fluorescens* genomes respectively in culture media. B- Lane 1, 100bp DNA Ladder. B-Lanes 2, 3 and 4, PCR products of ACC deaminase genes from *P. putida* treated culture media with Velum

© ICAS 2023

prime in 5µL and 10µL, 15µL / (10mL) concentrations respectively. B-Lanes 5, 6 and 7, PCR products of ACC deaminase gene from transconjugant *P. fluorescens* genomes treated in culture media with Velum prime in 5µL, 10µL and 15µL / (10mL) concentrations respectively. B-Lane 8, PCR products of ACC deaminase gene which was provided from a PhD. research study used as a positive control.

According to the results of Vantex and Velum prime pesticide treatments, there were intensive mutations in both bacterial genomes, and therefore, there were unavailable of ACC deaminase genes in the most of the treatments. The reasons behind of that are most pesticides have ability to affect non-target organisms, and consequently they cause the production of free radical (ROS) (Pandey et al., 2011). ROS is capable of fragmentations and oxidation DNA and proteins as well as DNA damaging as a consequence. Thus, DNA damaging causes to occur DNA mutation, and different alterations on the nucleotide bases will be occurred as well such as; 5,6-dihydroxycytosine, thymine glycol, 2,6-diamino-4-hydroxy-5-formamidopyrimidine and 8-hydroxyguanine (Evans et al., 2004). Furthermore, it was mentioned that pesticides have ability to impact on the molecular and biochemical actions of the cells which cause to prevent DNA repair enzyme activations and eventually decreasing the ability of DNA repairing process (Shimura-Miura et al., 1999). Therefore, treated bacterial genomes were probably mutated by Vantex and Velum prime pesticides, and subsequently ACC deaminase genes were unavailable in their genomes.

Conclusion

It is concluded from this study that the pesticides have differential effects on the growth of *Pseudomonas sp*., and their action vary at different sites. Indication has been observed that the pesticides (Topsin, Vantex and Velum prime) which were under laboratory and field condition possibly due to its high toxic nature reduced the population of these bacteria. Their direct effect on studied bacteria was a decrease in the number of viable bacterial population, and high indirect effect was a reduction in the availability of ACC deaminase gene in studied *P. putida* and tranconjugant *P. fluorescens* species. While, the other pesticide (Glyphosate) did not illustrate any observable effects on the viable counts of the tested bacteria, whereas in high doses might have effects on the genomes of transconjugant *P. fluorescens* in soil.

References

AL-ANI, M. A., HMOSHI, R. M., KANAAN, I. A. & THANOON, A. A. Effect of pesticides on soil microorganisms. Journal of Physics: Conference Series, 2019. IOP Publishing, 072007.

ALDEHAMEE, M. H. M. 2015. Study the effect of Topsin (Thiophanate-Methyl) fungicide on E. coli bacteria. *Al-Qadisiyah Journal of pure science,* 20**,** 77-84.

COOLS, H. J. & HAMMOND-KOSACK, K. E. 2013. Exploitation of genomics in fungicide research: current status and future perspectives. *Molecular plant pathology,* 14**,** 197-210.

DICK, R. E. & QUINN, J. P. 1995. Control of Glyphosate uptake and metabolism in Pseudomonas sp. 4ASW. *FEMS Microbiology Letters,* 134**,** 177-182.

© ICAS 2023

DUNFIELD, K. E., SICILIANO, S. D. & GERMIDA, J. J. 2000. The fungicides thiram and captan affect the phenotypic characteristics of Rhizobium leguminosarum strain C1 as determined by FAME and Biolog analyses. *Biology and Fertility of Soil,* 31**,** 303-309.

ESWARA, P. J. & RAMAMURTHI, K. S. 2017. Bacterial cell division: non-models poised to take the spotlight. *Annual review of microbiology,* 71**,** 393.

EVANS, M. D., DIZDAROGLU, M. & COOKE, M. S. 2004. Oxidative DNA damage and disease: induction, repair and significance. *Mutation Research/Reviews in Mutation Research,* 567**,** 1-61.

FIRDOUS, S., IQBAL, S. & ANWAR, S. 2020. Optimization and modeling of Glyphosate biodegradation by a novel Comamonas odontotermitis P2 through response surface methodology. *Pedosphere,* 30**,** 618-627.

GALLORI, E., CASALONE, E., COLELLA, C., DALY, S. & POLSINELLI, M. 1991. 1, 8-Naphthalic anhydride antidote enhances the toxic effects of captan and thiram fungicides on Azospirillum brasilense cells. *Research in microbiology,* 142**,** 1005-1012.

GRUBE, A., DONALDSON, D., KIELY, T. & WU, L. 2011. Pesticides industry sales and usage. *US EPA, Washington, DC*.

HIBBERD, M. C., WU, M., RODIONOV, D. A., LI, X., CHENG, J., GRIFFIN, N. W., BARRATT, M. J., GIANNONE, R. J., HETTICH, R. L. & OSTERMAN, A. L. 2017. The effects of micronutrient deficiencies on bacterial species from the human gut microbiota. *Science translational medicine,* 9**,** eaal4069.

KAUR, K. & KAUR, R. 2018. Occupational pesticide exposure, impaired DNA repair, and diseases. *Indian journal of occupational and environmental medicine,* 22**,** 74.

KEON, J. P., WHITE, G. A. & HARGREAVES, J. A. 1991. Isolation, characterization and sequence of a gene conferring resistance to the systemic fungicide carboxin from the maize smut pathogen, Ustilago maydis. *Current genetics,* 19**,** 475-481.

KWIATKOWSKA, M., RESZKA, E., WOŹNIAK, K., JABŁOŃSKA, E., MICHAŁOWICZ, J. & BUKOWSKA, B. 2017. DNA damage and methylation induced by Glyphosate in human peripheral blood mononuclear cells (in vitro study). *Food and Chemical Toxicology,* 105**,** 93-98.

LIMA, J. Y., MOREIRA, C., FREITAS, P. N. N., OLCHANHESKI, L. R., PILEGGI, S. A. V., ETTO, R. M., STALEY, C., SADOWSKY, M. J. & PILEGGI, M. 2020. Structuring biofilm communities living in pesticide contaminated water. *Heliyon,* 6**,** e03996.

MAHMUD, K. K. & KHUDHUR, A. M. 2022. Effects Of The Transconjugant Pseudomonas Fluorescens Carrying 1-Aminocyclopropane-1-Carboxylate Deaminase Gene On Drought Tolerance Of Wheat. *NVEO-NATURAL VOLATILES & ESSENTIAL OILS Journal| NVEO***,** 1227-1242.

MAHMUD, K. K.,(2022). *Inducing Wheat Drought Tolerance through Inoculation with Mutant Pseudomonas fluorescens*. (Doctoral dissertation).

© ICAS 2023

MÅRTENSSON, A. 1992. Effects of agrochemicals and heavy metals on fast-growing rhizobia and their symbiosis with small-seeded legumes. *Soil Biology and biochemistry,* 24**,** 435-445.

MELO, C. A., MASSENSSINI, A. M., PASSOS, A. B. R., CARVALHO, F. P., FERREIRA, L. R., SILVA, A. A. & COSTA, M. D. 2017. Isolation and characteristics of sulfentrazone-degrading bacteria Part B Pesticides, food contaminants, and agricultural wastes.

MUTURI, E. J., DONTHU, R. K., FIELDS, C. J., MOISE, I. K. & KIM, C.-H. 2017. Effect of pesticides on microbial communities in container aquatic habitats. *Scientific reports,* 7**,** 1-10.

PANDEY, A. K., NAGPURE, N., TRIVEDI, S. P., KUMAR, R. & KUSHWAHA, B. 2011. Profenofos induced DNA damage in freshwater fish, Channa punctatus (Bloch) using alkaline single cell gel electrophoresis. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis,* 726**,** 209-214.

QIU, D., KE, M., ZHANG, Q., ZHANG, F., LU, T., SUN, L. & QIAN, H. 2022. Response of microbial antibiotic resistance to pesticides: An emerging health threat. *Science of The Total Environment***,** 158057.

REASONER, D. J. 2004. Heterotrophic plate count methodology in the United States. *International journal of food microbiology,* 92**,** 307-315.

REN, N., ATYAH, M., CHEN, W.-Y. & ZHOU, C.-H. 2017. The various aspects of genetic and epigenetic toxicology: testing methods and clinical applications. *Journal of Translational Medicine,* 15**,** 1-13.

REVELLIN, C., LETERME, P. & CATROUX, G. 1993. Effect of some fungicide seed treatments on the survival of Bradyrhizobium japonicum and on the nodulation and yield of soybean [Glycine max.(L) Merr.]. *Biology and Fertility of Soil,* 16**,** 211-214.

RUSSELL, R. J., SCOTT, C., JACKSON, C. J., PANDEY, R., PANDEY, G., TAYLOR, M. C., COPPIN, C. W., LIU, J. W. & OAKESHOTT, J. G. 2011. The evolution of new enzyme function: lessons from xenobiotic metabolizing bacteria versus insecticide-resistant insects. *Evolutionary Applications,* 4**,** 225-248.

SHIMURA-MIURA, H., HATTORI, N., KANG, D., MIYAKO, K. I., NAKABEPPU, Y. & MIZUNO, Y. 1999. Increased 8-oxo-dGTPase in the mitochondria of substantia nigral neurons in Parkinson's disease. *Annals of neurology,* 46**,** 920-924.

STENERSEN, J. 2004. *Chemical pesticides mode of action and toxicology*, CRC press.

SUDHAKAR, P., CHATTOPADHYAY, G., GANGWAR, S., GHOSH, J. & SARATCHANDRA, B. 2000. Effect of common pesticides on nitrogen fixing bacteria of mulberry (morus alba L.). *Indian Journal of Agricultural Research,* 34**,** 211-216.

SZYMAŃSKA-CHARGOT, M., CYBULSKA, J. & ZDUNEK, A. 2011. Sensing the structural differences in cellulose from apple and bacterial cell wall materials by Raman and FT-IR spectroscopy. *Sensors,* 11**,** 5543-5560.

© ICAS 2023

TAIWO, L. & OSO, B. 1997. The influence of some pesticides on soil microbial flora in relation to changes in nutrient level, rock phosphate solubilization and P release under laboratory conditions. *Agriculture, ecosystems & environment,* 65**,** 59-68.

ZAREI, T., MORADI, A., KAZEMEINI, S. A., AKHGAR, A. & RAHI, A. A. 2020. The role of ACC deaminase producing bacteria in improving sweet corn (Zea mays L. var saccharata) productivity under limited availability of irrigation water. *Scientific reports,* 10**,** 1-12.

© ICAS 2023